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Studies on the artificial seed production of the sea urchin, *Strongylocentrotus intermedius* I. The growth of early juveniles in winter

(Ezobafun Uni Jinko Shubyo Seisan no Kenkyu I. Tokikan ni okeru Teisei Shoki Chi-uni no Seicho)

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in Hokusui Shikenpo (Scientific Report of the Hokkaido Fisheries Experimental Station), 1989, 33, pp. 21 - 29

The early juveniles of the sea urchin, *Strongylocentrotus intermedius* were reared in the outdoor and indoor tanks with no-warmed running sea water and metamorphosed in October, November and December, 1986. The growth of these early juveniles in the following months was as follows:

1) When the juveniles were reared in the outdoor tanks at a water range of 1-15℃, the juveniles that had metamorphosed in October grow about 7㎜ in mean test diameter but those that had metamorphosed in December reached about 1㎜ in the next April.

2) The growth pattern of the juveniles metamorphosed in October had a marked difference in the outdoor and indoor tanks. The juveniles reared in the outdoor tanks grew more rapidly than those in the indoor tank. Allmost all reached above 5㎜ in test diameter.

3) The sea water temperature appears to have a great influence on the growth of early juveniles, espcially those under 2㎜ in test diameter.

4) The variation in size frequency distribution of rapidly growing juveniles was small.

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Foreword

Studies on the artificial production of the Ezobafun sea urchin (*Stongylocentrotus intermedius*; abbreviated herefater as Si : translator) seed started with the basic research conducted by Nakamura et al [1], and were later conducted everywhere in Hokkaido [2-4]. Active research started in 1982 at the Hokkaido Institute of Mariculture. By 1985, a series of technical developments had been achieved, and the target was set on large-scale seed production [5]. Furthermore, in order to produce seed on a commercial basis from 1986, one

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developed production processes aimed at lowering the costs. The most expensive stage in the production of seed is the raising of juvenile sea urchins from the time they settle to the time when their shell diameter reaches 5 mm, which is when they start their medium growth. We used juveniles sea urchins collected in the fall (seed collected in October, November, December), to perform rearing test from 1986 to 1987 without heating, and gained some information regarding the relationship between the temperature of the rearing water and the growth of the juvenile sea urchins. Our findings are reported hereunder.

The present study was a joint study conducted by the Hokkaido Institute of Mariculture and the Shikabe Branch of the Hokkaido Institute for the Promotion of Mariculture. For the rearing from the state of floating larva to that of juvenile sea urchin, we used the installations of the Shikabe Branch. We received much cooperation from the personnel of the same branch with regard to the rearing of the floating larvae and the juvenile sea urchins, the measurements of the water temperature and the like. We thank all members sincerely.

Materials and method

We performed the floating-period rearing of the Si juvenile sea urchins used in the tests by the method commonly followed [6] at the Hokkaido Institute of Mariculture, that is, we allowed the juvenile sea urchins to settle on 60 x 30 cm, 0.3 mm thick corrugated plastic plates (made of polycarbonate) over which Ulvella lentes had been allowed to multiply. To vary the rearing water temperature, we carried out the settling on October 8, 9, on November 17, on December 17, 1986, and obtained juvenile sea urchins. In the present article,

we will divide them into the October, November and December groups and denote them hereafter in the same fashion. We show in Table 1 the Is floating-larva and the juvenile sea urchin rearing stages. Except for a part of the October group, we used parent sea urchins from the Sea of Japan.

The juvenile sea urchins having settled on the corrugated plates were held in 7.5 t FRP water tanks (1.5 x 10 x 0.5 m) and reared in a current of

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sand-filtered seawater. There were 7 tanks, that is, 2 outdoor and 1 indoor tanks for the October group, one outdoor and one indoor tank each for the November and December groups. Each group of collected seed was reared for 10 days in an indoor settling tank after being allowed to settle. Thereafter they were moved to indoor or outdoor rearing tanks. We provided no particular feed, but allowed the sea urchins to feed on the attached diatoms and Ulvella lentes multiplying on the corrugated plates.

Every month, we measured the shell diameter of the juvenile sea urchins. At the beginning, we measured 50-100 individuals in every tank. Later, we measured 50-100 individuals attached to the corrugated plates or to the walls of the tanks, upstream, midstream and downstream along the flow of water going through the same tanks, the total being 150-600 individuals. We show the readings in Table 2. We obtained the coefficient of variation from the average values of the various groups of readings and from the standard deviations.

$$\text{coefficient of variation} = \frac{\text{standard deviation}}{\text{average value}} \times 100$$

We also estimated the days on which the average shell diameter reached 1 mm, 2 mm, 3 mm on the basis of the average shell diameter values on the various measurement days, and of the extents of growth during the periods in between. The water temperature was measured every day at noon.

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Results

1. Changes in water temperature

Fig. 1 shows the water temperature changes outdoors and indoors. The highest values during the rearing periods both outdoors and indoors were 15.0 ºC in late October. Although the indoor values remained later somewhat high, they kept dropping until late February. The lowest water temperature was

recorded on February 16. It was 1.0 ºC outdoors, and 1.6 ºC indoors. Thereafter, the outdoor temperature rose, remaining about 1 ºC higher than the indoor temperature. On some days in late April, 5 ºC was exceeded.

2. Growth of the juvenile sea urchins shell diameter

Fig. 2 shows the changes in the average shell diameter values of the juvenile sea urchins in the 7 tanks as well as the changes in standard deviation. In the October group settled in early October the shell diameter after one month was 0.9 - 1.1 mm, and indicated a daily growth of 19.0 - 24.7 µm. In the November group settled in mid November, the shell diameter after

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one month was 0.5, 0.6 mm, indicating a daily growth of 6.8, 9.1 µm. When comparing to the indoor October group which showed a somewhat greater growth, one obtained 1/2 - 1/3. In the December group settled in mid December, the growth after one month was 0.4, 0.5 mm, with a daily growth of 4.0 µm. Compared to the October indoor group which showed a somewhat greater growth, it was 1/5 - 1/9. Even when compared to the November group, it was about 1/3.

After two months, the shell diameter in the October group was 1.7 - 2.6 mm, showing a daily growth of 30.6 - 47.2 µm. In the November group, the shell diameter was 1.0, 1.1 mm, showing a daily growth of 14.5, 14.8 µm. Compared to the October group, it was 1/2 - 1/3. The shell diameter of the December group was about 0.6 mm, showing a daily growth of 3.0, 3.7 µm, and was 1/10 or less, as compared to the October group. Compared to the November group, it was 1/4 - 1/5.

Thereafter, similarly, the daily growths were several times higher. For the October group, they were 19.6 - 30.0 µm and 42.4 - 65.5 µm when compared after three and four months respectively. The daily growths of the November group were 8.2, 10.3 µm and 16.9, 24.5 µm. For the December group, the daily growths were 6.2, 8.0 µm and 15.2 and 19.2 µm.

3. Appearance rate of juvenile sea urchins with shell diameters of 5 mm or more

We show in Fig. 3 the rate of appearance in the October group, of juveniles with a shell diameter of 5 mm or more. Three months after the settling, juvenile sea urchins of 5 mm or more began to appear in early January at the rate of 1 - 2 %. After four months, that is, in early

February, the rate was 44.1 - 53.6 % in the outdoor tank, and 24.2 % indoors. After 5 months, that is, in early March, the rate was 76.5 - 77.5 % outdoors, and 33.0 % indoors. After six months, in early April, it was 93-94 % outdoors, and 21.7 % indoors, which shows that the growth tended to be better in the outdoor tanks.

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4. Relationship between the growth of the juvenile sea urchins and the water temperature

Table 3 shows the number of days needed for the average shell diameter to reach 1 mm, 2mm, 3mm in the October, November and December groups, as well as the average water temperatures and integrated water temperatures for each of these periods. The day on which the shell diameter reached 1 mm in the October outdoor group was November 6, that is, 29 days after the settling. For the indoor group, it was estimated to be November 10, that is, 33 days after the settling. During this time, the average indoor and outdoor water temperatures were 12.9 and 11.6 °C, and the integrated values were 364.5 and 382.8 °C - days. For the November outdoor group, it was the 59th day. For the indoor group, it was the 53rd day. The required length of time was about double that required for the October group. During this time, the average water temperatures were 9.1 and 6.8 °C, and the integrated values were 358.4 and 359.4 °C - days. For the December outdoor group, it was the 105th day and for the indoor group, it was the 103rd day. The required number of days was thrice that noted in the October group, and twice that for the November group. During this time, the average water temperatures were 3.7 and 3.6 °C, and the integrated values were 390.7 and 368.8 °C - days. During the periods in which the diameter shells reached 1 mm, the average water temperatures in the tanks fluctuated greatly between 12.9 - 3.6 °C in the respective tanks. Yet, the integrated values were close to 358.5 - 390.7 °C - days.

The numbers of days for the shell diameters to reach 2mm from 1 mm were 21 and 35 for the October outdoor and indoor groups respectively. They were 63 and 71 in the November group. The average water temperatures were 10.2 and 8.7 °c, 3.2 and 3.1 °C, and the integrated values 214.1 and 304.9 °C - days, 203.8 and 217.1 °C - days.

The number of days required for the shell diameters to reach 3mm from 2mm were 30 and 34 respectively in the October outdoor and indoor groups, and 24 in both the November outdoor and indoor groups. The average water

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temperatures were 5.9 and 4.7 ºC, 4.9 and 3.7 and the integrated values 176.6 and 160.6 ºC - days, 118.0 and 88.4 ºC - days.

Fig 4 shows the consolidated values. It is evident that as the average water temperature declines, more days are required for the shells to reach 1 mm after settling, and for the 1 mm shells to reach 2 mm. No such correlation between average rearing water temperature and number of required days was noted with regard to the shells growing from 2 mm to 3 mm.

5. Average shell diameter values and the coefficients of variation

In Fig. 5 we plotted for each measurement month the correlations between the average values of the juvenile sea urchins shell diameters in the three outdoor, indoor tanks of the October group and the coefficients of variation. The results are similar to those in Table 2 for the groups measured every month.

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The average shell diameter of the juvenile sea urchins in the outdoor tank No. 99 was about 1 mm in early November, and the coefficient of variation at the time was 11.9 %. In early December, the shell diameter was 2.6 mm and the coefficient of variation 16.2 %. In early January, the figures were 3.1 mm and 19.8 %. So the coefficient of variation tended to increase together with the shell diameter. In early February, we measured five groups of 100 individuals each. The average shell diameters of these respective groups were in the 4.6 - 5.8 mm range. The coefficients of variation were 18.4 - 26.1 %. The coefficient of variation tended to decrease as the average value increased. In early March, we measured 6 groups of 100 individuals each. The average shell diameters were 5.3 - 6.4 mm, and the coefficients of variation were in the 30.3 - 18.2 % range. Like in the February measurement results, the groups with large average values tended to have small coefficients of variation. In early April, the average shell diameter was 6.9 - 7.7 mm and the coefficient of variation 19.1 - 25.8 %, so that the coefficient of variation was smaller than in February - March.

The juvenile sea urchins in the outdoor tank No. 91 had smaller average shell diameter values than in the tank No. 99 of January, and the coefficient of variation tended to be large. However, the tendency was the same as for tank No. 99 in February - April. In both tanks, the coefficient of variation tended to increase together with the average values until the shell diameters

reached 3 - 4 mm. Thereafter, the coefficient of variation was stable within the 20 - 30 % range.

On the other hand, the coefficient of variation tended to be greater among the juvenile sea urchins of the indoor tanks than among those of the outdoor tanks. The average values and coefficients of variation of each of the February - April months were evidently different from those of the two outdoor water tanks.

Discussion

The growths of the adult, larval, later bottom juvenile Si sea urchins has been elucidated through rearing experiments and such [7-11]. Yet, because of the difficulty of collection under natural conditions, little is known about the early bottom juvenile sea urchins from the time they settle to that of their growth to about 5 mm [12]. Meanwhile, one may say that it is important for seed production to know the growth conditions of the early bottom juvenile sea urchins as the information is needed to develop rearing techniques conducive to cost cutting.

It is known that generally the shell of Si grows from the winter to the spring and that the 0 - 1 year old larval sea urchins grow during the whole year [7]. The commonly held view is that the water temperature or feed does not directly affect the growth of the body [9]. However, the results of the present study confirmed that the growth of settled sea urchins with a shell diameter of about 2 mm is rather strongly affected by the water temperature. It takes juvenile sea urchins settled during the low-temperature period of December until the following spring to reach a shell diameter of 1 mm, whereas the juvenile sea urchins having settled during the high water temperature period of October reached during the same length of time a shell diameter of 7 - 8 mm or more. The juvenile having settled in November stand midway between the two above groups. The juvenile sea urchins having gone through the high water temperature period after settling until reaching 1 - 2 mm grow steadily in a short time. On the other hand, juvenile sea urchins at the low water temperature period and at the same stage needed a fairly long time to grow.

If one considers these facts in terms of integrated water temperature, we note that 359.4 - 390.7 °C - days are needed for the shell diameter to grow to 1 mm after settling, and that 203.8 - 217.1 °C - days (excepting the 304.9

°C - days of the October indoor group) are needed for the shell diameter to grow from 1 mm to 2 mm. Furthermore, 88.4 - 176.6 °C - days are needed for the shell to grow from 2 mm to 3 mm. The integrated water temperatures required for the shells to grow to 1 mm after settling time and for the shells to grow from 1 mm to 2 mm are respectively fairly similar, but we note some scattering among the integrated values with regard to shell growth from 2 mm to 3 mm, and the relationship between the water temperature and the feeding days was unknown. Although the water temperature affects strongly the shell growth to 2 mm after settling, it would seem that it has relatively little bearing once the shell diameter exceeds 2 - 3 mm.

In the present study, we made no detailed observations of the types or quantities of attached diatoms used as feed by the juvenile sea urchins, but there did not appear to be any shortage of feed for the juvenile sea urchins, at least up to the time their shells reached a diameter of 3 mm.

In fish culture, there appear during rearing time a small number of especially large individuals called "tobi", and they are sometimes regarded as important with regard to management strategy [13]. With regard to the production of seed intended for release, the main objective is to know how to produce a large quantity of seed with little scattering. In the present instance, by comparing the average shell diameter values and the coefficients of evaluation, we noted that the coefficient of variation increased as growth proceeded faster even though the coefficient of variation grew together with the growth. The juvenile sea urchins tended to be uniform. When comparing the outdoor and indoor tanks of the October group, we noted that the growth was clearly faster in the outdoor tank, and that there was little scattering. We discovered that regardless of the winter, rearing in outdoor tanks was preferable for the growth of the juveniles and for the production of seed free of much scattering. We did not know the reasons for the difference between the growths of the outdoor and indoor juvenile sea urchins, but it might be found in differences between the types of diatoms attached to the corrugated plates outdoors and indoors, and to differences in feed nutritive values.

The above facts show that the rearing of early bottom Si juvenile sea urchins collected in the fall is possible outdoors in unwarmed seawater, which contributes to lowering the costs of seed production. One will however have to develop techniques for lowering the production costs still further, which is also true for spring-collected juvenile sea urchins.

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Tables and Graphs

Table 1 Growing stages of the larval and juvenile Si sea urchins

a. fertilization date, b. --th month, c. day, d. parent sea urchin production site, e. Shakotan, f. Hiroo, g. Kumaishi, h. tank for larval rearing, i. settling date, j. juvenile sea urchins, k. tank No., l. tank location, m. indoors, n. outdoors

Table 2 Number of individual juvenile sea urchins measured and the collection sites

a. water tank No., b. after -- month(s), c. 100 individuals upstream, d. same, e. 50 individuals upstream, midstream, downstream, f. 100 individuals each upstream, midstream, downstream and at two places on the wall surface, with a total of 500 individuals, g. (same as e., but for 100 individuals), h. (same as f., but for total of 600 individuals)

Figure 1 Water temperature fluctuations in indoor and outdoor rearing tanks

a. water temperature, b. year, c. indoor tank, d. outdoor tank

Figure 2 Growth of the shell diameters of the juvenile sea urchins

a. shell diameter, b. year, c. outdoors, d. indoors, e. --th month group

Figure 3 Appearance rate of juvenile sea urchins with shell diameters of 5 mm or more in the October group

a. frequency, b. year, c. --th month, d. outdoor tank, e. indoor tank

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Table 3 Numbers of days needed for the average shell diameter of juvenile sea urchins to reach 1 mm after settling, 2 mm from 1 mm, 3 mm from 2 mm, the average water temperature values (ºC) during this time and the integrated values (ºC - days)

a. collected seed group / shell diameter, b. settling, c. outdoors, d. indoors, e. --th month group, f. day, g. indicates date

Figure 4 Relationship between the average rearing water temperature and the number of days needed for the average shell diameter to reach 1, 2, 3 mm

a. number of days, b. average water temperature (ºC), c. day(s), d. outdoors, indoors, e. settling, f. shell diameter

Figure 5 Relationship between the average shell diameter values for each group measured in the October group and the coefficients of variation (the figures denote the measurement months)

a. coefficient of variation, b. average value, c. indoor tank, d. outdoor tank

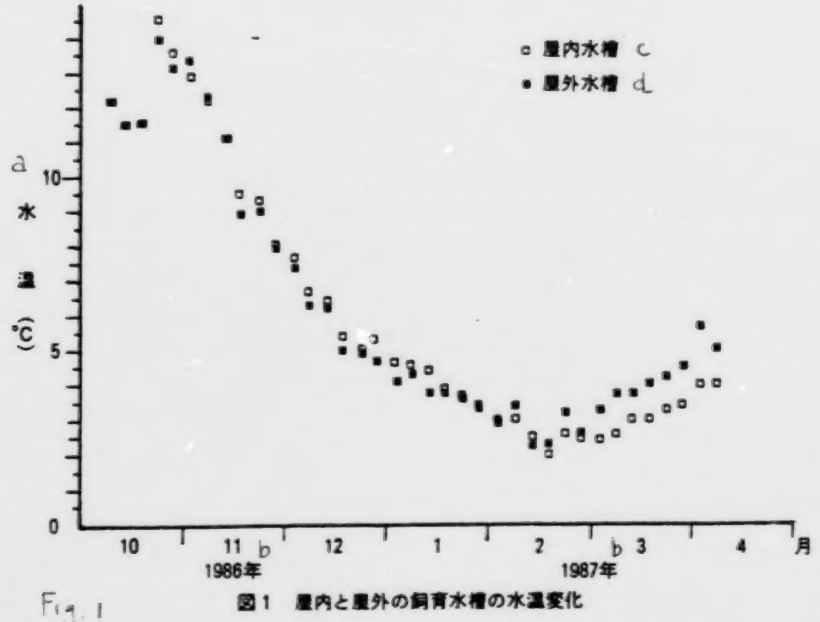

Fig. 1 図1 屋内と屋外の飼育水槽の水温変化

Table 1　表1　エゾバフンウニの幼生および稚ウニの飼育経過

a	受精月日	9月16日c			10月27日c			11月28日c					
d	親ウニ産地	e積丹町		f広尾町	g積丹町			積丹e町、熊石町g					
h	幼生飼育槽	0.5 t		0.5 t	1.0 t		0.5 t	1.0 t		0.5 t			
i	沈着月日	10月8日、9日			11月17日			12月17日					
j	稚ウニ	水槽番号K	b	c	c	b	c	b	c				
			106	99	91	93	107	95	104				
		場所l	屋内	屋外	屋外	屋外	屋内	屋外	屋内				
			m	n	n	n	m	n	m				

Table 2　表2　稚ウニの測定個数と採集か所

a 水槽番号	91、99、106	93、107	95、104
1か月後b	上流で100個体 c	上流で100個体 c	上流で100個体 c
2か月後b	同上d	同上d	上、中、下流で各50個体 e
3か月後b	同上d	上、中、下流で各50個体 e	同上d
4か月後b	上、中、下流、壁面2か所で各100個体ずつの計500個体 f	同上d	同上d
5か月後b	上、中、下流、壁面2か所、底面で100個体ずつの計600個体 h	上、中、下流で各100個体 g	−
6か月後b	同上d	−	−

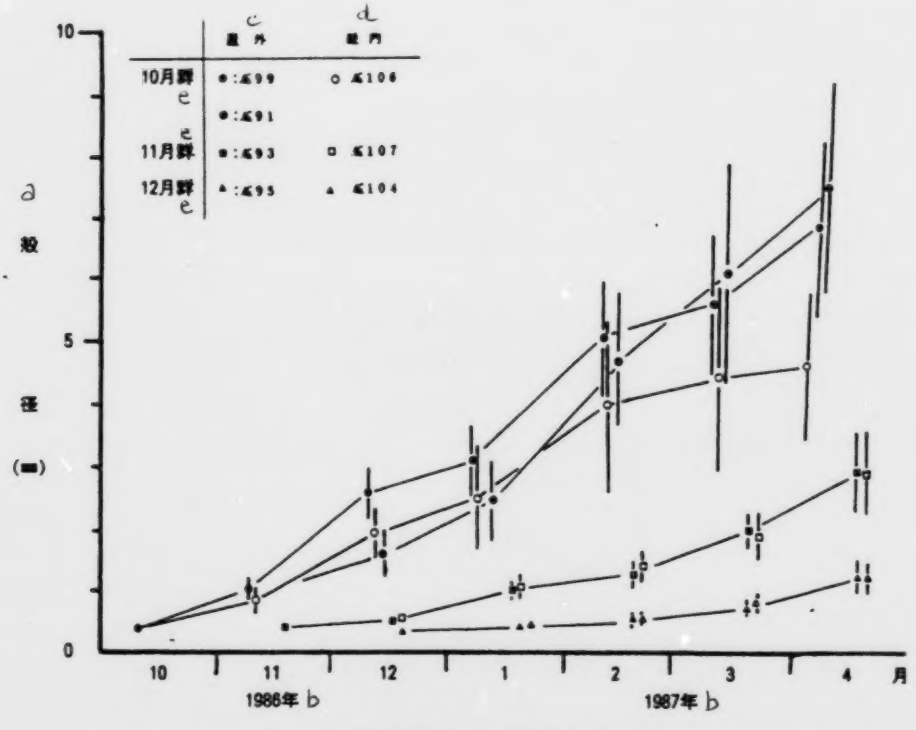

Fig. 2　図2　稚ウニの殻径の成長（平均値と標準偏差）

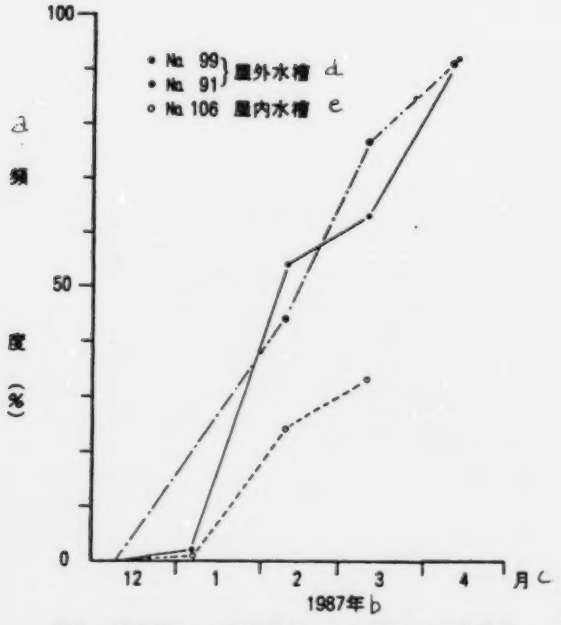

Fig. 3　　図3　10月群における殻径5㎜以上の稚ウニの出現率

Table 3　　表3　稚ウニの平均殻径が沈着～1㎜、1～2㎜、2～3㎜となるのに要した日数とその期間の水温の平均値(℃)と積算値(℃・日)

a

採苗群＼殻径		沈　着 b	1 ㎜	2 ㎜	3 ㎜
e 10月群	c 屋外	29 日 f (10／ 8)　12.9℃ 364.5℃・日 f	21 (11／ 6)　10.2 214.1	30 (11／27)　5.9 176.6	(12／27)
	d 屋内	33 (10／ 8)　11.6 382.8	35 (11／10)　8.7 304.9	34 (12／15)　4.7 160.6	(1／18)
e 11月群	c 屋外	59 (11／ 7)　9.1 358.4	63 (1／15)　3.2 203.8	24 (3／19)　4.9 118.0	(4／12)
	d 屋内	53 (11／ 7)　6.8 359.4	71 (1／ 9)　3.1 217.1	24 (3／21)　3.7 88.4	(4／14)
e 12月群	c 屋外	105 (12／17)　3.7 390.7	(4／ 1)		
	d 屋内	103 (12／17)　3.6 368.8	(3／30)		

g ( ) は月日を示す

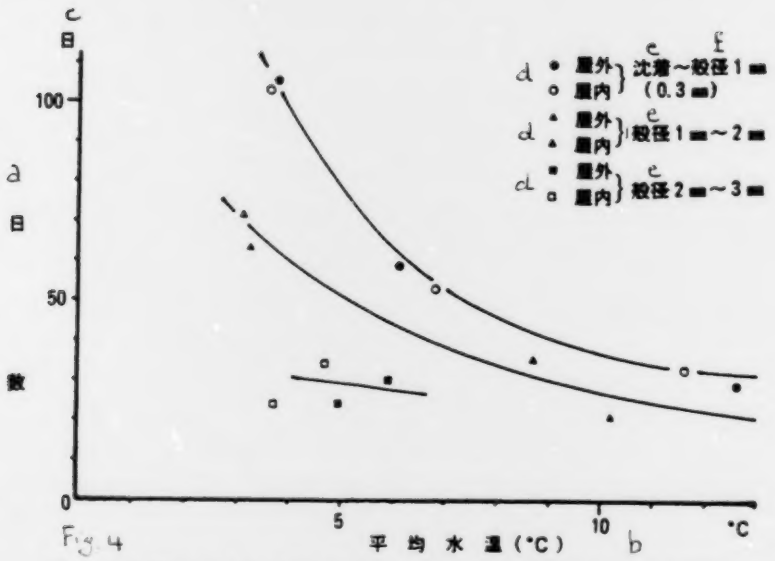

図4　飼育平均水温と平均粒径が1、2、3㎜となるのに要した日数との関係

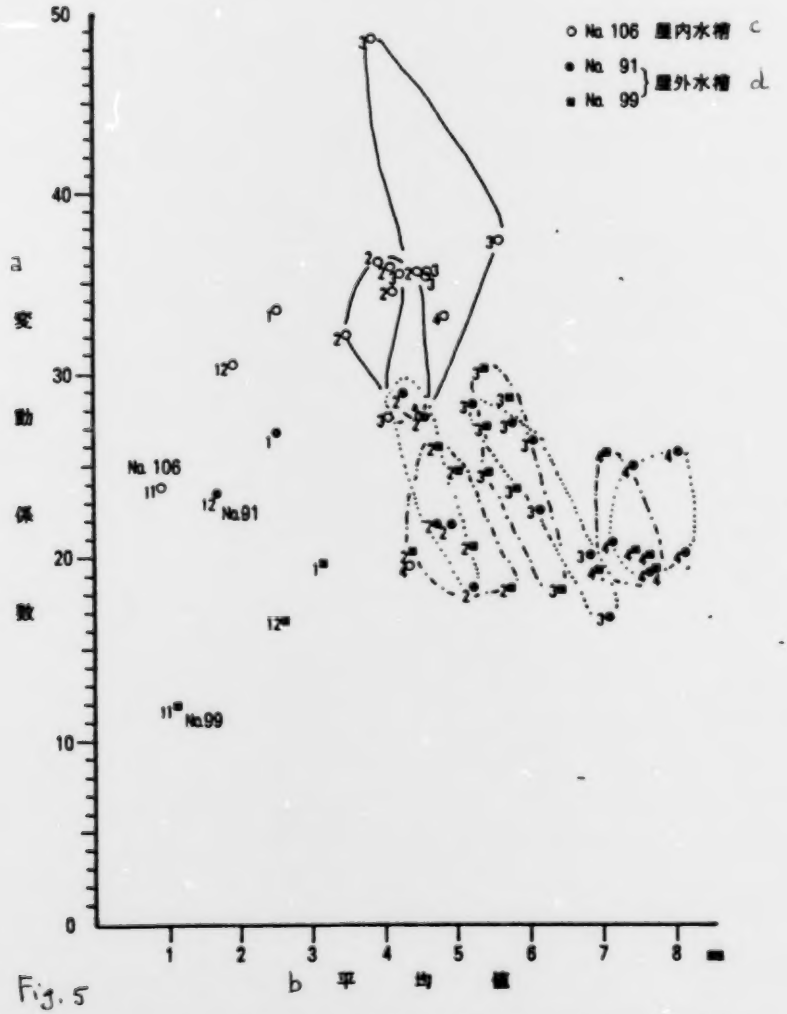

図5　10月群の測定群毎の粒径の平均値と変動係数との関係（数字は測定した月を示す）

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